COMPARATIVE STUDY OF PHYTOCHEMICAL EXPLORATION AND ROLE OF WILD SHRUB *LANTANA CAMARA* & *LANTANA INDICA* IN THE CONSERVATION OF OVER-EXPLOITED SPECIES.

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INTRODUCTION

Kingdom Plantae is one of the major and largest groups of all kingdoms. They are eukaryotic, autotrophic having complex cell structure. All plants have been proven as medicinal plants and hence they are known as medicinal herbs. Since a long back, the use of medicinal plants is in tradition just because of their medicinal properties towards the health issues of the big proportion of human population, and it is now most prominent in developing countries. Large numbers of plants are in medical practice and have been more than 3000 years, in Chinese, Ayurvedic and Unani traditional. We all are very well aware with the traditional systems of medicines and the role played by them in meeting the global health care needs. They are continuing to do so at present and shall play major role in future also. Almost, 75-80% of world's population, depends on the folk medicines, they have considered as primary health care method (Mitra*et al*., 2011).

In addition to medicinal uses, these plants are also used as edible goods, as a source of oil, feed, fuel and wood that has been long documented (Ray *et al*., 2010). Today, the herbal medicines are widely popular with allotropic medicines for the following reasons:

Go to cause and remove it.

Freedom to turn to different specialists.

Healing for many stubborn diseases.

Easy availability of medicines from natural sources.

The chemicals isolated from plants have significant role in the herbal as well as other medicines. The drugs derived from plants have played a central role in the health care of ancient and modern culture. Almost 60% of the drugs which are of natural origin are approved for cancer treatment. Vincristine, etoposide, Texans and camptothecins are the examples of plant anti-cancer compounds (Das *et al*., 2011). Medicinal plants are the major source of natural products having anti-metastatic and anti-proliferative properties (Serasanambati *et al*., 2015). Medicinal plants are rich source of secondary metabolites like tannins, alkaloids and flavonoids which are directly related to their phytochemical properties (Sarla *et al*., 2011). Active constituents now days are isolated from almost all the plants. Trade of these medicinal plants increases at faster rate. So, it is important to bring certain information about these plants and their valuable constituents.

So, keeping in view the entire above contest, it is more important to study the various species of genus *Lantana* L. An invasive plant species known to be among top 10 world's weeds. Alien plants are distributed in all the parts of the world, genus *Lantana* is among one of them. *Lantana* considered as the highest ranked species particularly invasive species in Queen's Island Australia (Sharma *et al*., 2005).

Classification

KINGDOM: - Plantae
SUBKINGDOM: - Tracheophyta
SUPERDIVISION: - Spermatophyta
DIVISION: - Magnoliophyta
CLASS: - Magnoliopsida
SUBCLASS: - Asteridae
ORDER: - Lamiales
FAMILY: - Verbenaceae
GENUS: - *Lantana* L.

Lantana native to south America. This genus was discovered by the great scientist Linnaeus in 17th century mainly in 1753.this genus includes more than 150 species. Carl Linnaeus, for the first time identify and record seven different species of *Lantana* in his species plantarum and found six species native to America and one species from Ethiopia. This species is mainly the hybrid species and commonly known as wild sage, red sage. It is mainly used as ornamental plant bought in India from Srilanka in 18th century. In India it is mainly distributed in Central Himalayas in the north and to the Southernmost part of India and hasdisplaced several native species (Kannan *et al*., 2012). After the discovery of this genus, it is distributed in all over the world that includes different countries like Africa, Australia, China, Cambodia, Srilanka etc. This hairy shrub found in India, and flowering occur during July to September (Singh, 2019). Commonly it is grown as a hedge plant but now consideredas major weed which needs an eradication (Natarajan and Ahuja, 1956). As *Lantana* is one of the most invasive species, this species has caused huge economic loss by its allelopathic as well as affecting nourishment, human health and ecological integrity of the native species (Mungi *et al*., 2020). I occur widely as troublesome weed in waste and cultivated lands. This plant swiftly distributed the natural equilibrium by growing in a very fast rate, but thisdoes not mean that this plant is not useful. This plant has high medicinal value. In ethnobotany, this plant used as sudorific, intestinal antiseptic, diaphoretic, anti-asthma, abdominal disorders, fever, cancer, catarrhal infection, chicken pox, eczema, hypertension, malaria, measles, swelling, rheumatism, tetanus, ulcers and many more. This genus also shows properties like antipyretic, antithrombin, antimalaria, insecticidal, antimicrobial, nematocidal, immunosuppressive, anti-inflammatory (Ali *et al*., 2017). This genus known to unveil the different microbial and bacterial activities and prove to be an important plant that shows antimicrobial and antifungal properties (Srivastava *et al*., 2015).

REVIEW OF LITERATURE

Distribution

Genus *Lantana* comes under the family Verbenaceae which includes 150 species. Genus *Lantana* is first discovered by Linnaeus in 17th century nearly in 1753 after its discovery this genus is highly distribute among 60 countries that includes Africa, Australia, China, Cambodia, south America, srilanka, India and many ore. Although this genus is native to south America and Ethiopia. This species being highly invasive species. it is mostly native to subtropical and tropical America but some of the species are also found in the region of tropical Asia and Africa in fact these areas have their indigenous species. The species is native of Central and South America and the Caribbean with geographical expansion between 35^{o}N and 35^{o}S (Priyanka and Joshi, 2013). There are different theories regarding the entities of these genus, this genus varies from 50 to 270 specific and sub specific entities, but it appears that a better estimate is around 150 species (Ojha and Dayal, 1992) (Sharma *et al*., 2005) (Manda and Seru, 2015) (Negi *et al*., 2019). In 18th century the European powers moved the plants from America to Europe and from Europe to their respective countries as this plant had great ornamental value, but little did, they know that it will become the most invasive species. In early 18th century, 1807 onwards these plants brought to India and after this the plant spread across the country due to the favorable climatic condition this plant got in India it is now a great threat to many indigenous species and its widespread also causing great impact in our native plant population. About 14 species of *Lantana* are believed to have been introduced into India. (Kannan *et al*., 2013). In 1807 this plant was brought first at the National Botanical Garden, Calcutta as an ornamental hedge plant. But later this plant spread and can easily be spotted in all open area's roadsides, railway tracks, edges of crop fields and open forests all over the country (Negi *et al*., 2019).

Morphological aspects

Lantana, also known as wild sage, Bunch berry, Ach man, Angel lipes, big sage. It is a deciduous shrub with the average height of 2m (6ft.) which bears thorns and multi-stems . Stemis square in shape which remains covered with bristly hairs when green, often armed or with scattered small prickles. *Lantana* have strong roots i.e., strong root system, they are so strong that after a repeated cuttings give new flush of shoots. The plant bear simple and opposite leaves with long petiole. Lamina of the leaf is oval and has rough and hairy and blunt toothed margins. Strong aroma is found in the leaves of the plants of the said genus. Its flowers are multi-colored, bear stalk and small. They are dense at the top and found in clusters with a corolla having narrow tube with four short spreading lobes. After anthesis, the colour of the flower change. These flowers occur in cluster which includes whit- pink-lavender or yellow-orange-red mix. The yellow coloration of the flower provides visual cue to pollinators and change in color and turning purple and finally to blue-black color. However, the berries are very poisonous in nature though these are attractive to insects and birds. Seed germination is easy and faster in *Lantana*. Different varieties of this genus are very difficult to differentiate based on morphological features. Some of them have re curved prickles some having their stems less prickly or even unarmed, some are less vigorous in growth, and some spread vigorously. Some varieties set seeds less freely. And

there are these dwarf varieties also which exclusively used as hanging baskets and as a hedge along borders of the houses (Natarajan and Ahuja, 1956) (Sharma *et al*., 2005) (Kannan *et al*., 2012) (Priyanka and Joshi, 2013) (Negi *et al*., 2019).

Cytological aspects

Multiples of 11 is considered as the basic number of this group i.e., Genus *Lantana*. Different species have different ploidy, but the basic number is constant for all the species for instance L. *camara* showing 2n = 33, 44, 55 respectively. Many researchers also have reported the presence of diploid and triploid i.e., 2n= 22, 33. Whereas another variety L. trifolia have chromosome number 2n = 48. Also, there is hexaploidy i.e., 2n =66 also reported. Meiosis of this genus shows that in diploid 2n=22, the chromosome number pair normally forming 11 bivalents. Triploids shows an occurrence of 5 to 8 trivalent, 3 to 9 bivalents and 1 to 7 bivalents in different combination. Some of the tetraploids shows normal 22 bivalent formed, while others show 2 to 3 quadrivalent, 2 to 6 trivalent, 7 to 16 univalent, 10 to 12 bivalents, 1 to 8 trivalent, 2 to 4 quadrivalents, 1 to 3 pentavalent and occasionally 1 heptavalent are seen in hexaploidy (Natarajan and Ahuja, 1956) (Chatha and Bir, 1988) (Czarnecki *et al*., 2014). As per Henderson (1969), in Australia, two forms of trailing *Lantana* had been reported and these both the forms were dramatically different in pollen viability and ploidy level/chromosome number. The weedy form was tetraploid with $2n = 4x = 48$ chromosomes and had ≈65% pollen stainability whereas the garden form was a triploid with $2n = 3x = 36$ chromosomes and had extremely low pollen stainability (less than 6%) and. In India, only the triploid trailing *Lantana* was noticed (Raghavan and Arora, 1960).

Phytochemical profile

The investigation for entry vector microbial properties using pathogenic species bacteria includes *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyrogens, Pseudomonas aeruginosa, Salmonella typhi, Alcaligene faecalis, Bacillus subtilis, Micrococcus luteus, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumonia, , Micrococcus roseus, Proteus mirabilis, Escherichia coli and* fungi *(Aspergillus fumigatus, A, flavus, A. niger, Candida albicans and C. tropicals).* Genus *Lantana* shows allopathic effects and this activity of plant tend to reduce the vigor of other species in its proximity (Priyanka and Joshi., 2013). In 2015 the studies conducted by Manda and Seru on the *Lantana indica* shows the significant anti-ulcer activity at all tested dose level on induced gastric ulcer in albino rats, these activities were dose related and the extract that has been used in this study is aqueous extract and ethanolic extract having dose level 200 mg per kg.

Ali *et al*., 2017 conducted phytochemical investigation of *Lantana* with methanol extract of leaves and isolated three new chemical constituents in it. These chemicals were characterised n-heneitriacont-4-en- 16β, 17β, 18β-triol (n-heneitriacontenol, 3), 6,4'-dimethoxy-7, 3'- dihydroxyflavone-7-o- β-D-glucopyranoside (*Lantana*flavone 7-o-β-D-

glucoside), the structure of all the isolated phyto constituents have been established on the basis of spectral data analysis and chemical reaction. In 2019, Desh Deepak Singh conducted antimicrobial activity on this plant against clinical isolates of human pathogen and found that *Lantana indica* was quite effective against most of the microbial species investigated, therefore characterization of various extract may be useful in identification of some noble compound having less toxicity. Recent studies in 2020 by Palei *et al*., shows that the presence of some functional group capping the AgNPs UV -vis spectrophotometer shows absorbance peak in the range of 436-446 nanometre, it can be concluded that the green synthesis of AgNPs from *Lantana camara* extract is easy, fast, one step, eco-friendly and alternative conventional method. the synthesized AgNP preparations from *Lantana camara* leaf extract show potential for application as broad-spectrum antimicrobial agent.

Pharmacological insights

Traditionally, the society is using this plant just because of its medicinal properties like-sudorific, intestinal antiseptic, diaphoretic and to treat asthma, abdominal disorder, bilious fever, cancer, catarrhal infections, chicken pox, eczema, hypertension, malaria measles, swelling, rheumatism, tetanus and ulcers. it works as a suppressor against anti-microbial, insecticidal, antitumor antimalarial, anti-inflammatory, immunosuppressive, anti-thrombin, antipyretic and antiseptic activities, Studies conducted by Ali *et al.* (2017) also shows that *Lantana* is being a noxious weed has several minor uses mainly in herbal medicines. Several studies and researches conducted on the exploitation of chemical constituents present in different parts of plant species, the extract from the leaves can be employed to combat antimicrobial, fungicidal, insecticidal its potential to serve as bio side has also been illustrated in several researchers. (Priyanka and Joshi; 2013). In 2010 the study led by Venkataswamy *et al*., discovered that the antimicrobial and preliminary phytochemical properties of *Lantana* is high in the aqueous and organic solvent extract from the leaves of *Lantana indica* when tested against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyrogens*, by Agar well diffusion method the results showed promising antibacterial activity against the tested bacteria. In 2019 the study shows that there is important species of *Lantana* that produce toxic compounds in the roots of, used as herbal medicine for the treatment of chicken pox, measles, asthma, skin itches, antiseptic for wounds and externally in leprosy.

KNOWLEDGE GAPS

As per the various review and research related articles it is more important to discuss here that lot of work have already done throughout the world on genus *Lantana*. But still there are huge research gap which need to be fulfilled. As per our knowledge is concerned there is not even a single combined report related to the part basis phytochemical analysis and antimicrobial analysis i.e., different alkaloids, vitamins, proteins, amino acid, minerals etc. on the basis of various analytical tool that is HPLC and GCMS. Not even a single

comparative report on the different fruits and flowers of distinct species of plant from

different regions of Uttar Pradesh and Himachal Pradesh. So, in this research will add more information in research and development for future researchers. The combined as well as comparative research report regarding these selected species of genus *Lantana* based on genetic markers biochemical activities and herbal preparation from Uttar Pradesh and Himachal Pradesh is still lacking therefore to fulfil this huge gap following objectives have been selected for the future research**.**

OBJECTIVES

1. Collection of germplasm of *Lantana camara* and *Lantana indica*.
2. Identification of both germplasms (morphotype/cytotype/chemotype) based on their cytomorphological and phytochemical analysis.
3. To study in-vivo/in-vitro biochemical activities of elite germplasms.
4. Molecular characterization to find out genetic diversity/variability of both species of *Lantana*.
5. Compilation and submission.

EXPECTED NEW KNOWLEDGE AND OUTCOMES

The germplasm of *Lantana* in Uttar Pradesh and Himachal Pradesh that is northern India exhibit a lot of cytomorphological and phytochemical variability which remains undocumented. The present work has been planned to assess the cytomorphological and phytochemical characterization resulting in identification of an elite germplasm, which will eventually help in identifying best germplasm for commercial significance.

Chemical and genetic characterization of germplasm of these species is of great significance for future programs of quality improvement of medicinal herbs. In order to maintain, evaluate and use germplasm efficiently, it is important to investigate the extent of genetic and phytochemical diversity available because we know that phytochemical variability depends upon the magnitude of various factors such as genetic variability.

The use of specific bioactive markers in the present study is expected to help in the identification and chemical characterization of the elite germplasm whose biochemical activities will be studied further.

Although lots of scientific work have already been done on the selected species but there are many knowledge gaps related to the scientific findings in these species which are yet to be discovered.

TECHNICAL PROGRAMME

The below mentioned experiments will be conducted as accordance with the selected objectives and would be planned to complete in our own laboratory at IIMT university.

Objective 1: - Collection of germplasm of *Lantana camara and Lantana indica* from various parts of Himachal Pradesh and Uttar Pradesh and its maintenance in the herbal cum Botanical Garden of IIMT university.

Methodology: - Germ plasm of *Lantana* will be collected from different eco geographic region of Himachal Pradesh and Uttar Pradesh India. this collection will be given collection number and grown in herbal cum botanical garden of IIMT university, proposed time period for this objective is 1 month.

Objective 2: - Identification of elite germplasm based on the morphological and phytochemical analysis of both species.

Methodology: - Characters such as plant height, root length, size of leaves, Berry colour etc. will be recorded from fresh material in the field to investigate the extent of morphological variability in the germplasm of collected accessions for cytochemical psychological analysis floral buds of suitable size will be collected from plant and will be fixed in cornoy's fixative for 24 hours and then preserved in 70% alcohol number of chromosomes will be counted by analysing PMC photo micrographs were taken for temporary preparations (Iqbal and Datta, 2005). Different plant part extract like root, fruit, leaves, flower and stem will be used and qualitative analysis of these extracts will be carried out by various HPLC, GC-MS methods, time proposed for this objective is 2 months.

Objective 3: - To study the in vivo and in vitro biochemical activities of both species.

Antioxidant activity

The antioxidant activity will be studied based on various in vitro assays (enzymatic and non-enzymatic) which includes radical scavenging assay, metal chelation assay and hydrogen and electron donating assays, time proposed for this activity is 1 month.

Antimicrobial activity

Different extract of various plants species will be screened for different pathogenic bacterial and fungal strains by disc diffusion essay as described by Perez and Co-worker in 1990 with certain modifications, time proposed for this activity is 1 month.

Anti-cancer activity

MTT assay method will be used to study the anti-cancerous activity on different cell lines. cancer cell lines will be arranged from authorised institution and cultured with some modification in standard protocol (Jhah *et al*., 2012), time proposed for this objective is 2 months.

Objective 4: - Molecular characterization will be done to find out genetic diversity and variability of selected medicinal plants.

Methodology: - Different available markers will be employed to study the genetic diversity. Various statistical methods/analysis will be applied for getting better results and to correlate these results with statical manner, time proposed for this activity is 1 month.

REFERENCES

Ali M., Jameel M., Mir S.R and Sultana S., (2017). Chemical constituents from the aerial parts of *Lantana indica* roxb. I*nternational Journal of Pharmacognosy*; Vol. 4(6): 193-199.

Chatha G. and Bir S., 1988. Cytology and distribution pattern of woody species of Verbenaceae in Palni hills. *Proceedings / Indian Academy of Sciences*, 98(2), pp.139-148.

Czarnecki, D., Hershberger, A., Robacker, C., Clark, D. and Deng, Z., 2014. Ploidy Levels and Pollen Stainability of Lantana camara Cultivars and Breeding Lines. *HortScience*, 49(10), pp.1271-1276.

Henderson R.J.F., (1969). A cytological study of *Lantana montevidensis* (Spreng.) Briq. Vol. 5: 1143-1149.

Jhah A.K., Jha M. and Kaur J. (2012). Ethanolic extracts of *Ocimum sactum, Azadirachta indica, Withania somnifera* cause apoptosis in SiHa cells. R.J.P.B.C.S., 3(2): 557-562.

Kannan R., Shackleton C. and Uma Shaanker R., 2012. Reconstructing the history of introduction and spread of the invasive species, Lantana, at three spatial scales in India. *Biological Invasions*, 15(6), pp.1287-1302.

Kattadiyil B.J., (2020). Impact of IBC on the real estate industry icsi iip, an ipa of ibbi, New delhi. *International journal of multidisciplinary educational research*, e-ISSN: 9876-9881.

Manda R.M & Seru G. (2015). Evaluation of *Lantana indica* for anti-ulcer activity in experimental rats. *International Journal of Pharmacy and Biological Sciences*, e-ISSN: 2230-7605.

Mazumder P., Das S., Das S. and Das M., 2011. PHYTO-PHARMACOLOGY of Berberis aristata DC: A REVIEW. *Journal of Drug Delivery and Therapeutics*, 1(2).

Mitra D., Saumya D., Sanjita D., Kumar D.M., (2011). Phyto-pharmocology of *Berberis aristata. Journal of drug delivery and therapeutics* Vol. 6: 568-572.

Mungi N., Qureshi Q. and Jhala Y., 2020. Expanding niche and degrading forests: Key to the successful global invasion of Lantana camara (sensu lato). *Global Ecology and Conservation*, 23, p.e01080.

Natarajan A.T., and Ahuja M. R., (1956). Cyto-taxonomical studies in the genus *Lantana*. Botany Division, Indian Agricultural Research Institute, New Delhi Vol. 11: 1137-1142.

Negi G., Sharma S., Vishvakarma C.R., Samant S., Maikhuri R.K., Prasad R.C. and Palni L.P., (2012). Ecology and Use of *Lantana camara* in India. *The Botanical Review* e-ISSN: 7845-7850.

Ojha B.M and Dayal N. (1992). Cytologicals Investigations in the Genus *Lantana* in India. *Tokyo Cytologia* 2 (9).

Palei N., Ramu S., Vijaya V., Thamizhvanan K., Balaj A. (2020), Green synthesis of silver nanoparticles using leaf extract of *Lantana camara* and its antimicrobial activity *International Journal of Green Pharmacy* Vol. 7: 2357-2362.

Perez C., Paul M. and Bazerque P. (1990). An anitibiotic assay by the agar-diffusion method. *Acta. Biol. Med. Exp.,15:* 113-115.

Priyanka and Joshi., (2013), Evaluation of *Lantana* against different microbes. *International Journal of Green Pharmacy*, Vol. 9: 56321-56326.

Raghavan, Arora R.S. (1960) Morphological and cytological studies in the genus *Lantana* L *Bull. Bot. Surv. India* 2: 299-303.

Ray R. and Gururaja K.V. (2011). Predictive distribution modeling for rare Himalayan medicinal plant *Berbneris aristata* DC. *Journal of Environmental Biology*, 32: 725-730.

Saini S. and Saini K.K (2020). *Lantana camera*: a problematic weed, *International Journal of Multidisciplinary Educational*, (2) 1462-1467.

Sarla V. and Sharma M. (2011). Effect of *Berberis aristata* on lipid profile and coagulation parameters. *African Journal of Pharmacy and Pharmacology*, 5(7): 943-947.

Serasanambati M., Chilakapati R. S., Manikoda P.K., Kanala R.J. (2015). Anticancer activity of methanolic extract of *Berberis aristata* in MCF-7 Human Breast Cancer Cell Lines. *International Journal of Life Sciences Biotechnology and Pharma Research,* 44: 31-35.

Shah M., Alharby H.F. and Hakeem K. H. (2020). *Lantana camara*: A Comprehensive Review on Phytochemistry, Ethnopharmacology and Essential Oil Composition. *Letters in Applied Nano Bioscience*, 1199 – 1207.

Sharma G.P, Raghubanshi A.S. and Singh J.S (2005). *Lantana* invasion: An overview. *Blackwell Publishing Asia Pty Ltd*, 3867-3872.

Singh D.D. (2019). Assessment of Antimicrobial activity against clinical isolates of human pathogen, Physiochemical and Phytochemical Analysis of *Lantana indica Rox Research J. Pharm. and Tech*; 12(8):3873-3877.

Srivastava M., Ahlawat S. and Lall A.M. (2015). Antimicrobial activity of a pep tide from the flowers of *Lantana indica* roxb. *An International Journal Society for Scientific Development*, 5672-5676.

Venkataswamy R., Doss A., Sukumar M. and Mubarack H., 2010. Preliminary phytochemical screening and antimicrobial studies of Lantana indica roxb. *Indian Journal of Pharmaceutical Sciences*, 72(2), p.229.